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Evaluation of novel forensic DNA storage methodologies

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ABSTRACT

An issue in forensic sciences is the secure storage of extracted DNA. Most of the time, DNA is frozen at −20 °C or −80 °C. Recently, new room temperature DNA storage technologies have been developed based on anhydrobiosis.

Two products use this technology: Qiasafe (Qiagen) and Gentegra (Genvault). In this study we focused on the recent Gentegra product and initiated a comparison versus −20 °C and Qiasafe storage. We compared the quantity and quality of DNA stored using anhydrobiosis technology against DNA stored at −20 °C, by performing STR profiling after short term storage. Furthermore, we studied the quantity and integrity of DNA after long term storage. Our results prove the high potential of this technology but it seems to be extraction dependent. Phenol/chloroform extracted DNA could be stored using the Gentegra matrix for more than 6 months without any obvious degradation. However, DNA extracted using magnetic beads could not be safely stored over the same period. Adaptations are therefore required to store this kind of samples.

1. Introduction

Success of forensic genetics has led to a considerable DNA storage issue. The most common storage method is freezing at −20 °C or −80 °C. With the increasing numbers of samples, freezing appears to be costly and not without risk of failure. To avoid these disadvantages, new room temperature DNA storage technologies have been developed. These new technologies are based on anhydrobiosis, a biological process employed by some multicellular organisms that are able to survive more or less complete dehydration [1]. In this system, the DNA solution is air-dried in a chemical medium to ensure its preservation over long periods of time. Chemicals form a protective and stable barrier to protect DNA against degradation and oxidation. DNA recovery is simply done by rehydration.

In an effort to find a methodology to store DNA at room temperature, Smith and Morin have tested trehalose [2]. Trehalose is one of the major compounds accumulated during anhydrobiosis [3,4]. Trehalose, with other compounds, replaces water and interacts with macromolecules during dehydration. Previous work had shown that trehalose retains activities of dry enzymes for days [5]. The work of Smith and Morin indicated that drying DNA in presence of trehalose is a good alternative to freezing. DNA could be stored without loss of amplifiable DNA for up to 1 year [2].

Two companies, Qiagen and Genvault, have developed DNA storage systems, respectively Qiasafe and Gentegra, using anhydrobiosis. The Qiagen product is based on a synthetic polymer that mimics the anhydrobiosis process. The Genvault product is an inert mineral medium which creates a water-free environment protecting DNA samples from hydrolysis. When air-dried in Gentegra medium, DNA becomes more stable at room temperature and less sensitive to UV light [6]. The aim of these products is to provide a sure and easy way to preserve DNA.

Both companies have performed internal studies using accelerating ageing by heating DNA sample. Heating of the DNA sample is well known to induce DNA damage [7]. This allows virtual years ageing in few weeks only. Qiasafe and Gentegra can protect DNA for, respectively, 30 and 10 accelerated years [8]. But this ageing does not mimic perfectly natural ageing.

A first study has underlined the high potential of this technology using the Qiagen and Gentegra conservation matrixes. These authors stored DNA for up to 3 weeks at room temperature without obvious degradation or loss [8]. However, this study presents some limitations. The authors did not document the ability of these conservation matrixes to undergo multiple cycles of hydration/drying nor did they test long term storage [8].

Here, we assessed the Gentegra conservation matrix using as references the −20 °C storage and the Qiasafe matrix. The first considered criterion was the DNA recovery. We treated several quantities of human DNA with the two conservation matrixes over multiple hydration cycles and assessed DNA quality by STR profiling. The second criterion was the integrity and quantity of DNA after storage at room temperature for 6 months.

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2. Materials and methods

2.1. DNA extraction

Blood samples were extracted using a standard organic extraction with phenol/chloroform followed by ethanol precipitation.

Buccal swabs DNA extraction was carried out using the Kingfisher robot (Thermo Electron, The Netherlands) using the NucleoMag96Trace kit from Macherey-Nagel (Germany) according to manufacturer's protocol.

2.2. DNA quantification

Quantification was performed using the Quantifiler system (Applied Biosystems, USA) and an Applied Biosystems 7500 Real-Time PCR system according to manufacturer's specifications. The quantification was done in duplicate. The average was considered for the results and the following experiments.

2.3. PCR amplification with Identifiler™ kit

The Identifiler™ kit (Applied Biosystems, USA) was used following manufacturer's recommendations. Twenty-eight cycles of reaction were performed. One ng of sample per reaction (according to Quantifiler quantification results) in a total reaction volume of 25 μl was amplified on a C1000 thermocycler (Bio-Rad laboratories, Belgium). Samples were analyzed on a 3130 XL Genetic Analyzer (Applied Biosystems, USA) using a 36 cm capillary array (Applied Biosystems, USA), POP-4 polymer (Applied Biosystems, USA), 1× genetic analysis buffer with EDTA (Applied Biosystems, USA), a 18 s injection at 1.2 kV and 15 kV electrophoresis. Two μl of PCR product were loaded with 10 μl of formamide (Applied Biosystems, USA). Genemapper ID v3.2 software (Applied Biosystems, USA) was used for analysis.

2.4. DNA application on the conservation matrix

The Gentegra (Genvault) and Qiasafe (Qiagen) conservation matrixes were used following manufacturer's specifications. Twenty μl of diluted sample was applied to the matrix, mixed gently by pipetting and dried overnight (16 h) under a chemical flow hood with a constant air humidity of 18%. The sealed tubes were store at 20 °C under a constant humidity of 18% in the dark. The humidity was measured using a hygrometer THG 312 (Oregon Scientific, USA). The quantities of DNA used for each experiment are reported in Section 3. Hydration of the dried samples mixed with the matrix was done with 20 μl of MilliQ water (Qiasafe) or the buffer purchased with the kit (Gentegra).

2.5. Hydration/drying cycles–freezing/thawing cycles

In the multiple hydration/drying cycles experiments, DNA mixed with the matrix was hydrated with 20 μl of MilliQ water (Qiasafe) or the buffer purchased with the kit (Gentegra) and then dried overnight as described above. Twenty-four hours were let between two cycles. Reference samples frozen at −20 °C were subjected to the same freezing/thawing cycles. Thawed samples were let at room temperature for 30 min and then frozen again at −20 °C.

2.6. Statistics

Comparison between conditions was done using a TTEST.

3. Results

3.1. DNA recovery

We first determined the efficiency of the DNA recovery after storage in the conservation matrixes. Per tube, 1 μg of DNA in 20 μl was loaded on the conservation matrixes and dried as indicated in Section 2. Twenty-four hours later, the matrix was hydrated in 20 μl, two aliquots were taken, one for quantification, one for STR profiling and stored until analyses at −20 °C. These steps were reproduced two more times (one cycle per 24 h). All samples were quantified twice, at the same moment, with frozen references to avoid variations between quantifications. Regardless of the precision of the real-time quantification, Fig. 1 shows a high recovery of the DNA for both Gentegra and Qiasafe matrixes. No significant change of DNA quantities was detected after the three cycles of hydration/drying except for the second cycle with the Qiasafe matrix.

The quality of the stored DNA was also investigated by STR profiling. To avoid experimental variations, all samples from the first and the last cycle of hydration/drying were freshly diluted to obtain 1 ng in 10 μl of MilliQ water and amplified simultaneously with the −20 °C DNA references. The matrix DNA samples and the corresponding −20 °C references were electrophoresed simultaneously.

With the three conservation methods (Gentegra, Qiasafe or −20 °C freezing), the STR profiles were complete and above a threshold fixed to 50 RFU. The intensity of the peak for each allele was compared to the intensity obtained for the same allele

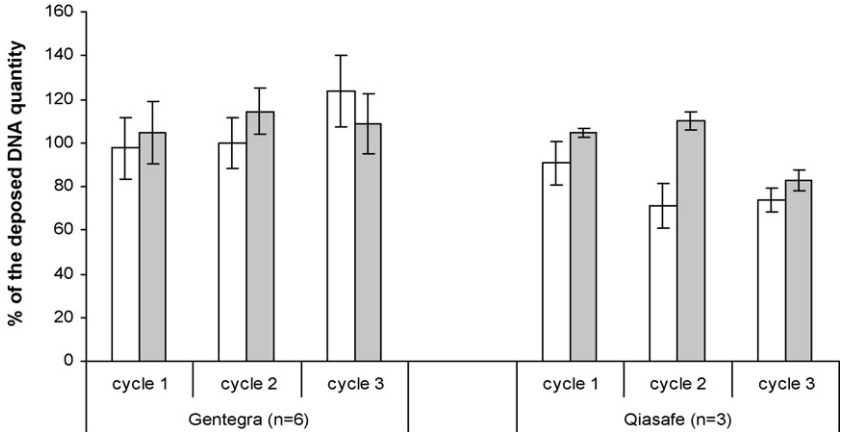

Fig. 1. Percentage of the original quantity of DNA stored using the Gentegra or the Qiasafe matrixes after one, two or three cycles of hydration/drying. Reference samples stored at −20 °C were submitted to three cycles of thawing/freezing of 30 min, one cycle per day. White columns: matrix storage; gray columns: −20 °C freezing.